DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/27/2010 has been entered.

Receipt is acknowledged of an amendment, filed 9/27/2010, in which claims 28, 29, 32-36, 59 and 65 were cancelled, and claims 24, 46-54, 56-58 and 60-64 were amended. Claims 24, 31, 37, 39, 40, 46-54, 56-58 and 60-64 are pending and under consideration.

Claim Objections

Claim 46 is objected to because of the following informalities:

While the metes and bounds of the claim are clear in light of the teachings of the specification, the wording of the phrase "a protein-expressing DNA fragment in which cDNA of a virus vector that has been constructed by inserting a coding gene of an arbitrary protein into an RNA virus is ligated to an inducible promoter which is induced by the transcription factor" is confusing. It would be remedial to amend the claim to insert commas around the phrase "that has been constructed by inserting a coding gene of an arbitrary protein into an RNA virus" to make it clear that the cDNA of a virus vector is ligated to an inducible promoter. Appropriate correction is required.

Response to Arguments - Claim Objections

The objection of claim 65 is moot in view of Applicant's cancellation of the claim in the reply filed 9/27/2010.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 57 and 64 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This is a new rejection.

Claim 57 recites "wherein the coding gene of an arbitrary protein is substituted with a gene that encodes a coat protein of the virus." If taken literally, the phrase would mean that the coding gene of the arbitrary protein is replaced with a coat protein gene. The independent claim specifically requires the presence of "a protein-expressing DNA fragment in which cDNA of a virus vector that has been constructed by inserting a "coding gene of an arbitrary protein into an RNA virus is ligated to an inducible promoter." Further, the specification states the following at page 38:

> It is preferable that a gene that encodes an arbitrary protein be inserted downstream of the promoter for a gene that encodes the coat protein of the virus, and more preferably be substituted with a gene that encodes the coat protein of the virus. With the gene inserted at these sites, there will be no production of the viral coat protein, and the amplified viral gene will not form particles and infect other plants, thereby solving the problem of viral spreading.

The specification does not redefine the phrase "substituted with" in the specification. In other instances the phrase is used with its conventional meaning (e.g., page 11, 33 and 35). In order to clarify the metes and bounds of the claim, it would be remedial to amend the claim to recite

"wherein the coding gene of the arbitrary protein replaces a coat protein gene so that there will be no production of the viral coat protein."

Claim 64 recites "A producing kit for performing the process for producing a plant culture cell for protein production as set forth in claim 46." The claim is composed only of a preamble stating the intended use of the kit without reciting a transitional phrase or body of the claim. The claim does not positively recite any elements that must be present in the kit. Many different products could be used in the method of claim 46, and the claim provides no indication as to which products must be present in the kit. For example, the kit could be composed of a plant culture cell and a culture medium. Alternatively, the kit could be composed of a DNA fragment of an inducible promoter linked to a virus vector into which a coding gene of an arbitrary protein has been ligated, and an inducer for the inducible promoter. Accordingly, the metes and bounds of claim are unclear.

The following is a quotation of the fourth paragraph of 35 U.S.C. 112:

> Subject to the following paragraph, a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claim 57 is rejected under 35 U.S.C. 112, fourth paragraph, as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form.

This rejection is based upon the literal interpretation of the phrase "wherein the coding gene of an arbitrary protein is substituted with a gene that encodes a coat protein of the virus." Claim 57 depends from claim 46, which requires "a protein-expressing DNA fragment in which cDNA of a virus vector that has been constructed by inserting a coding gene of an arbitrary

protein into an RNA virus is ligated to an inducible promoter." Thus, claim 46 requires the presence of a coding gene of an arbitrary protein." If claim 57 is interpreted as replacing the coding gene of an arbitrary protein with a gene that encodes a coat protein of the virus, then the coding gene of an arbitrary protein is no longer present, and claim 57 does not include all of the limitations of independent claim 46.

Claim 64 is rejected under 35 U.S.C. 112, fourth paragraph, as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form.

Claim 64 is drawn to "A producing kit for performing the process for producing a plant culture cell for protein production as set forth in claim 46." The claim is not a proper dependent claim, because the product claim can be infringed without infringing the base method claim, because the product can be used in a method other than that recited in claim 64.

Response to Arguments - 35 USC § 102

The rejection of claims 28 and 29 under 35 U.S.C. 102(b) as being anticipated by Garger et al is moot in view of Applicant's cancellation of the claims.

The rejection of claims 24, 31, 37, 39 and 40 under 35 U.S.C. 102(b) as being anticipated by Garger et al has been withdrawn in view of Applicant's amendment to the claims in the reply filed 9/27/2010.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 24, 31, 37, 39 and 40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Garger et al (US Patent Application Publication No. 2002/0061309 A1, cited in a prior action; see the entire reference) in view of Weber et al (Journal of Virology, Vol. 66, No. 6, pages 3909-3912, June 1992; see the entire reference) and Zuo et al (US Patent No. 6,452,068 B1, cited in a prior action; see the entire reference). This is a new rejection, necessitated by the amendment filed 9/27/2010.

Garger et al teach DNA fragments which are tobamovirus vectors modified to express fusion proteins (e.g., paragraph [0002]). Garger et al teach that vectors for the genetic manipulation of plants have been derived from several naturally occurring plant viruses, including tobacco mosaic virus (TMV), where the vector comprises a cDNA of a plant RNA virus (e.g., paragraphs [0005], [0006], [0050] and [0068]). Garger et al teach the recombinant

viral nucleic acid where a coding sequence of a protein of interest (arbitrary protein) is inserted downstream of a coat protein gene promoter and where the protein of interest is produced as a fusion protein comprising the coat protein (e.g., paragraphs [0002], [0008], [0057]-[0066]). Further, Garger et al teach that the expression of the coat protein fusion proteins may be driven by any of a variety of promoters functional in the genome of the recombinant plant viral vector, where the protein is expressed by a promoter 5' to the fusion protein encoding region (e.g., paragraph [0066]). Moreover, Garger et al teach that the expression of the fusion protein may be elevated or controlled by a variety of plant or viral transcription factors (e.g., paragraph [0066]). Garger et al teach recombinant viral nucleic acid vectors and plant cells comprising the recombinant nucleic acid vectors (i.e., transformants) (e.g., paragraphs [0010], [0070] and [0075]). Garger et al teach a collection of items (i.e., kit) comprising the DNA fragment and vector comprising the DNA fragment. Garger et al exemplify a plasmid vector comprising cDNA of a tobacco mosaic virus (TMV) that contains a coding sequence of a protein fused to the native coat protein coding sequence, where the 3' end of the viral cDNA is immediately followed by a self-processing ribozyme sequence from satellite tobacco ringspot virus RNA, and where the T7 promoter is 5' to the virus vector cDNA and ribozyme sequence (e.g., paragraphs [0002], [0008], [0010], [0050], [0059], [0068], [0077], [0100]-[0122]). The plasmid DNA taught by Garger et al (e.g., paragraphs [0100]-[0122]) would necessarily be capable of being incorporated into the genome of a plant cell by non-homologous recombination.

Garger et al do not teach the DNA fragment where the tobamovirus is specifically tomato mosaic virus. Garger et al do not teach the 5' promoter comprises 6xUASgal4, and do not teach the DNA fragment further comprising a GVG coding sequence.

Weber et al teach that tomato mosaic virus (ToMV) is closely related to tobacco mosaic virus, and like tobacco mosaic virus, has been used for many fundamental studies concerning packaging, replication, movement and host-pathogen interactions of plant viruses (e.g., page 3909, left column, 1st full paragraph). Weber et al teach that a major breakthrough occurred when full-length cDNA clones of ToMV were obtained, and the viral cDNA was fused to the lambda PM promoter, thereby allowing the generation of *in vitro* transcripts by E. coli RNA polymerase (e.g., page 3909, left column, 1st full paragraph). Weber et al teach a plasmid comprising a DNA fragment comprising the cDNA of the ToMV virus vector where the 35S RNA promoter from cauliflower mosaic virus (CaMV) or the T7 promoter was placed upstream of the cDNA of the virus vector to promote transcription instead of the lambda PM promoter (e.g., paragraph bridging pages 3909-3910; Figures 1 and 2). Weber et al teach that the plasmid comprising the 35S promoter and ToMV cDNA was infectious, and ToMV was readily observed in *Chenopodium quinoa* (e.g., page 3910, paragraph bridging columns; Table 1). Weber et al teach that the inoculation of plants with a plasmid containing a cDNA clone of an RNA virus under the control of a eukaryotic promoter seems to be a convenient alternative to the generation of *in vitro* transcripts and should facilitate the analysis of viral mutants generated at the DNA level.

Zuo et al teach a single vector comprising a promoter operably linked to a transcription factor and a promoter regulated by the transcription factor operably linked to a protein coding gene (e.g., column 9, line 27 to column 11, line 2). Zuo et al teach the vector where the transcription factor is a chimeric transcription factor in which the regulatory region of the rat glucocorticoid receptor (GR) is added to the DNA-binding domain of the yeast transcription factor GAL4 and the transactivating domain of the herpes viral protein VP16, where the chimeric

transcription factor is called GVG (e.g., column 9, lines 50-67). When the vector comprises the GVG transcription factor, the inducible promoter contains six copies of the GAL4 upstream activating sequence (UAS) (6xUASGal4, e.g., column 9, lines 50-67; Figure 1). Zuo et al teach another construct referred to as XVE, which is similar to the GVG system but contains the DNA binding domain of the bacterial repressor LexA and the regulatory region of the human estrogen receptor (e.g., column 10, lines 43-53). Zuo et al specifically teach that the XVE construct can be used in place of the GVG construct as long as the proper inducer is used for the construct being used (e.g., column 10, lines 48-51). When the vector comprises the XVE transcription factor, the inducible promoter contains eight copies of LexA binding sites fused to the 35S minimal promoter at -46 (O_{LexA}-46; e.g., paragraph bridging columns 20-21; Figure 13).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the DNA fragment, vector comprising the DNA fragment and plant cell (transformant) comprising the DNA fragment or vector of Garger et al to include the cDNA of the tomato mosaic virus vector taught by Weber et al, because Garger et al teach it is within the ordinary skill in the art to provide such compositions comprising a cDNA of a tobamovirus and exemplify the cDNA of a tobacco mosaic virus, and Weber et al teach that tomato mosaic virus is very similar to tobacco mosaic virus. Further, Weber et al exemplify plasmid vectors similar to those of Garger et al with respect to the DNA fragment comprising a promoter followed by the cDNA of a tobamovirus. Garger et al exemplify tobacco mosaic virus as the cDNA of a tobamovirus, and Weber et al exemplify tomato mosaic virus as the cDNA o f a tobamovirus. Moreover, Weber et al teach transformants comprising the plasmid vector comprising the DNA fragment comprising the cDNA of tomato mosaic virus.

One would have been motivated to make such a modification in order to receive the expected benefit of providing a DNA fragment capable of producing tomato mosaic virus in *Chenopodium quinoa* as taught by Weber et al. One would have been motivated to produce fusion proteins for use as vaccine antigens or vaccine antigen precursors in plant cells, to provide products capable of producing proteins free of both bacterial-related toxins and organisms or particles pathogenic to humans, as taught by Garger et al (e.g., paragraphs [0072] and [0004]). Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Furthermore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to further modify the DNA fragment, vector and plant cell (transformant) of Garger et al and Weber et al to replace the promoter upstream of the tomato mosaic virus cDNA with a promoter comprising 6xUASGal4 of Zuo et al, because Weber et al teach it is within the skill of the art to substitute one promoter for another (e.g., lambda PM promoter, 35S CaMV promoter, or T7 promoter). Furthermore, Garger et al teach that any promoter functional in a plant can be placed 5' to the fusion protein encoding region (e.g., paragraph [0066]), and Zuo et al specifically teach that the promoter comprising 6xUASGal4 is functional in a plant.

One would have been motivated to make such a modification in order to receive the expected benefit of providing regulatable expression of the coat protein fusion as suggested by Garger et al with the regulatable system taught by Zuo et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 46-48, 50, 51, 56-58 and 60-64 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mori et al (The Plant Journal, Vol. 27, No. 1, pages 79-86, 2001, cited on the IDS filed 3/31/2006; see the entire reference) in view of David et al (Plant Physiology, Vol. 125, pages 1548-1553, April 2001, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 9/16/2009 and is reiterated below.

For the purposes of this rejection, claim 57 has been interpreted as requiring the coding gene of an arbitrary protein to be present in the cDNA of the virus, and as requiring the replacement of the coat protein gene with the coding gene of the arbitrary protein.

Mori et al teach a process for producing a transformant for protein production, comprising (i) transforming *N. benthamiana* host cells with a GVG transcription factor-expressing DNA fragment in which the GVG coding sequence is operably linked to the CaMV 35S promoter; where transforming is done by an Agrobacterium method (ii) screening the transformants obtained in step (i) for an individual F0 plant expressing GVG; and (iii) crossing the F0 GVG-expressing plants with 2FR plants containing cDNA of a virus vector that has been constructed by inserting a coding gene of human gamma interferon (IFN) into an RNA virus, where the IFN coding sequence is ligated to the 6XUASGal4 inducible promoter, which is induced by the GVG transcription factor (e.g., page 82, *Production of transgenic plants containing cDNA of RNA1 or cDNAs of both RNA2 and FCP2IFN*; pages 82-83, *Induced replication of FCP2IFN and subgenomic mRNA amplification in GVG1 x 2FR plants*; page 85, *Transformation of Nicotiana benthamiana*; Figure 1). Specifically, the virus vector used in the method of Mori et al contains a cDNA of a Brome mosaic virus that has been constructed by inserting a coding sequence of a human gamma interferon (IFN) protein into the RNA virus, and

ligating a ribozyme sequence to the 3' end of the virus vector cDNA (e.g., page 85, paragraph bridging columns; Figure 1). The ribozyme sequence is a ribozyme sequence of satellite tobacco ringspot virus (e.g., page 85, paragraph bridging columns). In the virus vector, the IFN sequence was inserted in place of the coat protein gene (e.g., page 80, right column, 2nd full paragraph). Further, Mori et al teach a transformant produced by the abovementioned process, where the transformant produces IFN protein in the presence of dexamethasone (e.g., Figure 4). Mori et al teach that the GVG transcription factor has a property of being activated by the hormone dexamethasone, a synthetic steroid hormone (e.g., page 82, *Analysis of the accumulation of RNA1 in response to DEX treatment*). Mori et al teach the method where the virus vector originates in a virus that is a Brome mosaic virus, which is a single strand (+) RNA plant virus (e.g., page 80, right column, 2nd full paragraph). With regard to claim 64, a kit is a collection of items, and Mori et al teach at least one item for use in the process of producing the abovementioned transformant (e.g., page 85, Experimental procedures).

Mori et al do not teach the method where the GVG transformants are further transformed with the cDNA of a virus vector that encodes IFN using an Agrobacterium method. Further, Mori et al do not teach the method where the cells are tobacco BY-2 cells.

David et al teach that the tobacco (*Nicotiana tabacum*) BY2 cell line is well characterized, highly homogenous, and shows an exceptionally high growth rate (e.g., page 1548, left column, 1st paragraph). Further, David et al teach that BY2 cells can be easily transformed without the need for protoplast preparation and stable transgenic calli, and suspension-cultured cells are easily obtained (e.g., page 1548, left column, 1st paragraph). David et al teach a method that brings together the advantages of the BY2 cell line with the advantages of the tetracycline derepressible system (e.g., page 1548, right column, full paragraph). David et

al teach a method for producing a transformant for protein production, comprising (i) transforming BY2 cells with pBinTet1 vector, containing tetR under the control of the cauliflower mosaic virus (CaMV)-35S promoter; (ii) selecting clonal and stable transformants, named BY2-tetR, on kanamycin medium; (iii) and transforming the BY2-tetR cells with a pTX-Gus-int, a vector containing β-glucuronidase (Gus) under the control of the "Triple-Op" promoter coupled with CaMV 35S (e.g., paragraph bridging pages 1548-1549; page 1549, left column). David et al teach that Gus activity was induced in the BY2-tetR cells comprising pTX-Gus-int by the addition of AhTc (e.g., Figure 1). David et al teach that a high steady-state expression of tetR ensures an efficient repression of the "Triple-Op" promoter (e.g., paragraph bridging pages 1549-1550). David et al teach Agrobacterium-mediated transformation of the BY2 cells (e.g., page 1552, Cell Transformation).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Mori et al to include a first Agrobacterium-mediated transformation step of BY2 cells with the GVG expression vector, and a second Agrobacterium-mediated transformation step of BY2 cells with the IFN expression vector, as taught by David et al because David et al teach it is within the ordinary skill in the art to use BY2 cells for regulated expression of a protein product and Mori et al teach regulated expression of the IFN protein product.

One would have been motivated to make such a modification in order to receive the expected benefit of selecting for BY2 GVG transformants with desirable levels of GVG expression prior to transformation with the IFN expression vector, because David et al teach that desirable levels of tetR could be identified prior to the second transformation step. It would have been within the ordinary skill of the art to transform the BY2 cells based upon the teachings of

David et al, and it would have been within the skill of the art to screen for desirable levels of GVG by Northern blotting as taught by Mori et al. Furthermore, one would have been motivated to perform a second transformation step in BY2 cells rather than produce plants and cross the plants as taught by Mori et al in order to save time, because David et al teach that BY2 cells have an exceptionally high growth rate and are easy to transform. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 49 is rejected under 35 U.S.C. 103(a) as being unpatentable over Mori et al (The Plant Journal, Vol. 27, No. 1, pages 79-86, 2001, cited on the IDS filed 3/31/2006; see the entire reference) in view of David et al (Plant Physiology, Vol. 125, pages 1548-1553, April 2001, cited in a prior action; see the entire reference) as applied to claims 46-48, 50, 51, 56-58 and 60-64 above, and further in view of Zuo et al (US Patent No. 6,452,068 B1, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 9/16/2009 and is reiterated below.

The combined teachings of Mori et al and David et al are described above and applied as before.

Mori et al and David et al do not teach the method where the transcription factor is LexA-VP16-hER, the inducible promoter is O_{LexA}-46, and the inducer is estrogen.

Zuo et al teach the vector where the transcription factor is a chimeric transcription factor in which the regulatory region lf the rat GR is added to the DNA-binding domain of the yeast transcription factor GAL4 and the transactivating domain of the herpes viral protein VP16,

where the chimeric transcription factor is called GVG (e.g., column 9, lines 50-67). When the vector comprises the GVG transcription factor, the inducible promoter contains six copies of the GAL4 upstream activating sequence (UAS) (6xUASGal4, e.g., column 9, lines 50-67; Figure 1). Zuo et al teach another construct referred to as XVE, which is similar to the GVG system but contains the DNA binding domain of the bacterial repressor LexA and the regulatory region of the human estrogen receptor (e.g., column 10, lines 43-53). Zuo et al specifically teach that the XVE construct can be used in place of the GVG construct as long as the proper inducer is used for the construct being used (e.g., column 10, lines 48-51). When the vector comprises the XVE transcription factor, the inducible promoter contains eight copies of LexA binding sites fused to the 35S minimal promoter at -46 (O_{LexA}-46), and the inducer is estrogen (e.g., Example 12; Figure 13).

Mori et al and David et al both teach the use of regulatable transcription factors capable of being modulated for regulated expression of a protein. Mori et al specifically teaches the use of the GVG system, and Zuo et al specifically teaches that it was within the skill of the art to substitute the XVE system for the GVG system in order to achieve the predictable result of providing inducible expression of a protein. The XVE system comprises the claimed LexA-VP16-hER transcription factor, which is activated by estrogen, and the O_{LexA}-46 promoter.

Claims 52-54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mori et al (The Plant Journal, Vol. 27, No. 1, pages 79-86, 2001, cited on the IDS filed 3/31/2006; see the entire reference) in view of David et al (Plant Physiology, Vol. 125, pages 1548-1553, April 2001, cited in a prior action; see the entire reference) as applied to claims 46-48, 50, 51, 56-58 and 60-64 above, and further in view of Rasochova et al (US Patent Application Publication No.

2003/0074677 A1, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 3/3/2010 and is reiterated below.

The combined teachings of Mori et al and David et al are described above and applied as before.

Mori et al and David et al do not teach the method where the virus vector comprises tobacco mosaic virus.

Rasochova et al teach a vector comprising cDNA of an RNA virus vector that has been constructed by inserting an exogenous RNA component and a ribozyme sequence at the 3' region (e.g., paragraph [0049]). Rasochova et al teach the vector where the exogenous RNA component has a coding function in which the RNA acts as a messenger RNA, encoding a sequence which, when translated by the host cell, results in the synthesis of a peptide or protein (e.g., paragraph [0047]). Rasochova et al teach the vector where the virus vector originates in a virus that is a single strand (+) RNA virus, such as tobacco mosaic virus (e.g., paragraphs [0042], [0135], [0139] and [0141]). Rasochova et al teach the DNA molecule where the exogenous RNA component is inserted in place of the coat protein coding sequence (e.g., paragraphs [0057] and [0137]). Rasochova et al teach the use of the vector to make transgenic plants expressing the protein (e.g., paragraphs [0138]-[0141]). Further, Rasochova et al teach it is within the skill of the art to use an inducible promoter for the expression of the exogenous RNA component (e.g., paragraphs [0049]-[0050]).

Because Mori et al and Rasochova et al both teach vectors for the expression of a protein in plant cells, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the tobacco mosaic virus vector of Rasochova et al for the Brome mosaic virus vector of Mori et al, where expression of the protein is under the control of

the 6xUASgal4 promoter, in order to achieve the predictable result of providing a vector for the inducible expression of a protein in a plant cell.

Response to Arguments - 35 USC § 103

The rejection of claims 32-36 under 35 U.S.C. 103(a) as being unpatentable over Garger et al in view of Zuo et al is moot in view of Applicant's cancellation of the claims in the reply filed 9/27/2010.

The rejection of claims 32-34 and 65 under 35 U.S.C. 103(a) as being unpatentable over Garger et al in view of Martinez et al is moot in view of Applicant's cancellation of the claims in the reply filed 9/27/2010.

With respect to the rejection of claims 46-48, 50, 51, 56-58 and 60-64 under 35 U.S.C. 103(a) as being unpatentable over Mori et al in view of David et al, Applicant's arguments filed 9/27/2010 have been fully considered but they are not persuasive.

Independent claim 46 is drawn to a method comprising (i) a first transforming step of transfecting a plant culture cell with a transcription factor-expressing DNA fragment in which a coding gene of a transcription factor is ligated to a promoter for expressing the transcription factor; (ii) a screening step of screening plant culture cells obtained in the first transforming step, for an individual plant culture cell expressing the transcription factor; and (iii) a second transforming step of transfecting the individual plant culture cell, obtained in the screening step, with a protein-expressing DNA fragment in which a cDNA of a virus vector, that has been constructed by inserting a coding gene of an arbitrary protein into an RNA virus, is ligated to an inducible promoter which is induced by the transcription factor." The specification does not provide a limiting definition for the term "DNA fragment." The specification indicates that the

term DNA fragment broadly encompasses "at least..."cDNA of an RNA virus vector in which a gene that encodes an arbitrary protein to be produced in the cell has been inserted; and a ribozyme sequence bound to the 3' end of the virus vector cDNA." See page 37. The specification does not exclude DNA fragments which are plasmid DNA fragments.

At pages 7-9, the response discusses example embodiments disclosed in the specification. At page 8, the response states the following:

> Usually, a plasmid vector is employed in a plant such that an enhanced expression efficiency is obtained. Use of a virus vector, on the other hand, tends to cause a deterioration in an expression efficiency. As illustrated in FIG. 7 and other examples of the present application, an effect of example embodiments is higher expression efficiency of 60% (even using a virus vector) due to the ligation of the ribozyme sequence to the 3' end of the virus vector and the transformation step being performed in multiple stages.

First, it is noted that the claimed method does not exclude the use of a plasmid vector. Second, the working examples of the specification use a plasmid vector. Example 1 of the specification discloses the construction of expression vector pTA7001-ToMV-erG3(SF3), which is a plasmid vector based on the Ti plasmid pTA7001 (e.g., pages 57-58). In Example 2, presents the methods and results used in the experiment of Figure 7. In Example 2, the vectors used are plasmid vectors based on the Ti plasmid pTA7001, and the vectors contain the coding sequence for GVG, the 6XUASgal4 promoter, and the ToMV variant downstream of the 6XUASgal4 promoter (e.g., page 62). BY2 cells were transformed with a plasmid control vector (sold diamond, no ribozyme sequence), a plasmid vector comprising the ribozyme sequence of hepatitis delta virus (sold squares), and a plasmid vector comprising the ribozyme sequence of satellite tobacco ringspot virus (solid circles). The phrase "PRECULTURE PERIOD (IN DAYS)" means the number of days the cells were subcultured prior to steroid hormone treatment

(paragraph bridging pages 64-65). At pages 64-65, the specification describes the following conclusions based upon the results of the experiment shown in Figure 7:

> As in clear from Figure 7, with the control vector, the percentage of GFP-expressing cells was below 5% even on day 7. On the contrary, the percentage of GFP-expressing cells was about 25% in the vector containing the ribozyme sequence of hepatitis delta virus, and about 60% in the vector containing the ribozyme sequence of satellite tobacco ringspot virus.

Accordingly, the results of the experiment demonstrate that the ribozyme sequence of satellite tobacco ringspot virus provides higher levels of expression in BY2 cells when the ribozyme is covalently bound to the 3' end of a tomato mosaic virus cDNA. Contrary to the assertions made at page 8 of the reply, Figure 7 does not show that transformation being performed in multiple stages provides higher expression efficiency of 60%. The experiment was performed with a single transformation step. Thus, Example 2 is not a working example of the claimed invention, and the claims are not commensurate in scope with any unexpected improvement in expression efficiency that may have been obtained by using the ribozyme sequence of satellite tobacco ringspot virus in the particular system used in Example 2.

At pages 8-9, the reply discusses the two-step procedure used in Example 3 of the specification. The reply indicates that the example provides for "transforming steps at two different stages, which prevents or reduces the possibility of a protein becoming chimeric such that the expression of a function of the protein is improved."

It is unclear how the stable expression of a protein in the plant cell of Example 3 differs from that of the prior art. These arguments do not provide evidence of secondary considerations sufficient to overcome the rejection of record.

At the paragraph bridging pages 11-12, the response states the following:

> Conventionally, transformation of a plant by use of a virus vector has been difficult. That is, even in a case where the plant is transfected with multiple genes by using a single virus vector, expression efficiency of the multiple genes is very low. Such a problem does not arise if no virus vector is employed, i.e., if a vector other than a virus vector, e.g., plasmid, is employed. In other words, the problem arises selectively in a case where a virus vector is employed. Further, the problem is more serious in a case where a plant culture cell is transformed than in a case where a plant is transformed.

As stated in the reply, no problem arises if a plasmid is employed. Both Mori et al and David et al teach the use of plasmids, thus this problem is avoided by the prior art. Specifically, Mori et al teach the use of plasmids based on pTA7001 (e.g., page 85, *Plasmids*), and David et al teach the use of plasmids based upon pBin (e.g., page 1552, Constructs).

At page 12, the response asserts that an improved expression efficiency of 60% would not be achieved in the references cited as neither of the references insert a virus vector at two different stages as recited in independent claim 46.

This argument is not found persuasive. Claim 46 does not recite the insertion of a virus vector at two different stages. The claim requires the insertion of a DNA fragment at two different stages, where the second DNA fragment to be inserted comprises the cDNA of a virus. Both Mori et al and David et al teach two separate transformation steps: One transformation step to insert a vector expressing a transcription factor, and a second step to insert a vector comprising a promoter responsive the transcription factor and a coding sequence of a protein of interest (arbitrary protein of the claims). Mori produced transgenic *N. benthamiana* plants expressing GVG by using a binary vector pTA7001BB1, and, in a separate transformation step, Mori produced transgenic *N. benthamiana* plants comprising the cDNA of a virus vector comprising a coding sequence of a protein of interest by transforming using binary vectors pBICBPBR2R and o/bICHgCP2IFNR (e.g., page 82, left column). Mori et al do <u>not</u> teach a

single transformation step with a single vector comprising GVG and the cDNA of a virus vector comprising a coding sequence of a protein of interest. Furthermore, David et al teach two separate transformation steps in BY2 cells, where the cells were first transformed with pBinTet1 vector, encoding *tetR* transcription factor, and then the cells were separately transformed in a second transformation step with pTX-Gus-int, a vector containing Gus under the control of the "*Triple-Op*" promoter coupled with CaMV35, which is a promoter regulated by TetR (e.g., paragraph bridging pages 1548-1549; page 1549, left column, 1st full paragraph). Accordingly, the references do teach insertion at two different stages. Even if the 60% efficiency obtained in the specification was unexpected, it is not commensurate in scope with the claims. The experiment in which 60% efficiency was obtained involved a single transformation step. The specification indicates that the vectors used in the experiment are shown in Figure 5(A) and 5(B) (e.g., page 63). Those vectors contain both the GVG sequence and the inducible promoter linked to the cDNA of a virus comprising the coding sequence for GFP. Furthermore, the 60% efficiency was not obtained with any ribozyme sequence as claimed. The ribozyme sequence of hepatitis delta vector only provided about 25% efficiency (see the paragraph bridging pages 64-65 of the specification).

The response asserts that when transforming a plant by using a normal vector such as a plasmid, one skilled in the art would not be motivated to employ a time- and effort-consuming "serial transforming method" as the method of claim 46, especially when the efficiency of transformation in which a plant is transformed by using a single vector is satisfactorily high.

This argument is not found persuasive. As discussed above, both Mori et al and David et al use two transformation steps. David et al specifically teach a "serial transforming method" using plasmid DNA to transform BY2 cells in culture (e.g., paragraph bridging pages 1548-1549;

page 1549, left column, 1st full paragraph). The teachings of the references are not being modified in the rejection of record to separate a single transforming step into two transforming steps. One following the guidance of the references would have used two transforming steps. Further, David et al teach that BY2 cells can be easily transformed without the need for protoplast preparation and stable transgenic calli, and suspension-cultured cells are easily obtained (e.g., page 1548, left column, 1st paragraph).

At page 13, the response acknowledges that Mori et al and David et al teach two transforming steps. However, the response asserts that neither reference, nor the combination thereof, teaches inserting a transcription factor at a first step and then inserting a DNA fragment coding a virus vector at a second step, which allows for expression of a protein carried on a virus vector. These arguments are not found persuasive. Mori et al teach a process for producing a transformant for protein production, comprising (i) transforming *N. benthamiana* host cells with a GVG transcription factor-expressing DNA fragment in which the GVG coding sequence is operably linked to the CaMV 35S promoter; where transforming is done by an Agrobacterium method (ii) screening the transformants obtained in step (i) for an individual F0 plant expressing GVG; and (iii) crossing the F0 GVG-expressing plants with 2FR plants containing cDNA of a virus vector that has been constructed by inserting a coding gene of human gamma interferon (IFN) into an RNA virus, where the IFN coding sequence is ligated to the 6XUASGal4 inducible promoter, which is induced by the GVG transcription factor (e.g., page 82, *Production of transgenic plants containing cDNA of RNA1 or cDNAs of both RNA2 and FCP2IFN*; pages 82-83, *Induced replication of FCP2IFN and subgenomic mRNA amplification in GVG1 x 2FR plants*; page 85, *Transformation of Nicotiana benthamiana*; Figure 1). Specifically, the virus vector used in the method of Mori et al contains a cDNA of a Brome mosaic virus that has been

constructed by inserting a coding sequence of a human gamma interferon (IFN) protein into the RNA virus, and ligating a ribozyme sequence to the 3' end of the virus vector cDNA (e.g., page 85, paragraph bridging columns; Figure 1). The ribozyme sequence is a ribozyme sequence of satellite tobacco ringspot virus (e.g., page 85, paragraph bridging columns). In the virus vector, the IFN sequence was inserted in place of the coat protein gene (e.g., page 80, right column, 2nd full paragraph). Thus, Mori et al teach crossing two plants where one plant expresses the transcription factor and the other plant contains the virus vector where expression of the protein from the virus vector is regulated by the transcription factor. David et al teach a method for producing a transformant for protein production, comprising (i) transforming BY2 cells with pBinTet1 vector, containing tetR transcription factor under the control of the cauliflower mosaic virus (CaMV)-35S promoter; (ii) selecting clonal and stable transformants, named BY2-tetR, on kanamycin medium; (iii) and transforming the BY2-tetR cells with a pTX-Gus-int, a vector containing β-glucuronidase (Gus) under the control of the "Triple-Op" promoter coupled with CaMV 35S (e.g., paragraph bridging pages 1548-1549; page 1549, left column). Thus, David et al teach "serial transformation" of a plant cell culture, where the plant cells are first transformed with a vector encoding a transcription factor, and then the cells are transformed with a vector comprising a gene whose expression is regulated by the transcription factor. Furthermore, one would have been motivated to perform a first transformation step with a vector encoding GVG and a second transformation step in BY2 cells with the virus vector of Mori et al rather than produce plants and cross the plants as taught by Mori et al in order to save time, because David et al teach that BY2 cells have an exceptionally high growth rate and are easy to transform.

At pages 13-14, the response asserts that the Examiner does not provide a rationale to combine the teachings of Mori and David. The response asserts that the Examiner has merely

provided a conclusory statement that it would have been obvious to combine the references. The response does not consider the benefits cited by the examiner to be a rationale to combine the references. Specifically, the expected benefits of (i) selecting BY2 GVG transformants with desirable levels of GVG expression prior to transformation with the IFN expression vector and (ii) using BY2 cells, which have an exceptionally high growth rate and are easy to transform, are considered to be mere conclusory statements without any reasoning. Thus, the response asserts that the Examiner has not established a prima facie case of obviousness for combining Mori and David.

These arguments are not found persuasive. The Examiner has clearly set forth the rationale to combine the teachings of Mori et al and David et al:

> It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Mori et al to include a first Agrobacterium-mediated transformation step of BY2 cells with the GVG expression vector, and a second Agrobacterium-mediated transformation step of BY2 cells with the IFN expression vector, as taught by David et al because David et al teach it is within the ordinary skill in the art to use BY2 cells for regulated expression of a protein product and Mori et al teach regulated expression of the IFN protein product.
>
> One would have been motivated to make such a modification in order to receive the expected benefit of selecting for BY2 GVG transformants with desirable levels of GVG expression prior to transformation with the IFN expression vector, because David et al teach that desirable levels of tetR could be identified prior to the second transformation step. It would have been within the ordinary skill of the art to transform the BY2 cells based upon the teachings of David et al, and it would have been within the skill of the art to screen for desirable levels of GVG by Northern blotting as taught by Mori et al. Furthermore, one would have been motivated to perform a second transformation step in BY2 cells rather than produce plants and cross the plants as taught by Mori et al in order to save time, because David et al teach that BY2 cells have an exceptionally high growth rate and are easy to transform.

Both Mori et al and David et al teach that it is desirable to screen for transformants with desirable levels of the transcription factor transgene. Mori et al state, "One F0 GVG1 plant

(GVG1-7) with high-level accumulation of RNA1 after DEX treatment was obtained by screening the 24 F0 GVG1 plants by Northern blot analysis." See page 82, left column. David et al state, "The main constraint of this system is the preliminary selection of material expressing the TetR at a high level, a prerequisite for strict control of the expression of a gene under the control of the "*triple-Op*" promoter." See the paragraph bridging pages 1548-1549. Further, David et al state, "Our results show that BY2-tetR cell lines expressing tetR at a high level allow the production of a foreign protein in the cell under conditions of transient expression…On the basis of the results obtained in transient transformation, the BY2-tetR17 line was chosen for recipient cells." See page 1550, left column. In addition to obtaining BY2 cells with desirable levels of GVG expression, one would have been motivated to use the BY2 cells to save time. The use of BY2 cells doesn't require the growth of plants and crossing of the plants as taught by Mori et al. Combining the teachings of Mori et al and David et al results in a simple substitution of vectors used in the method of David et al with the vectors taught by Mori et al in order to obtain predictable results. Accordingly, the Examiner provided reasons to combine the teachings of Mori et al and David et al, with a rational underpinning to support the legal conclusion of obviousness.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

With respect to the rejection of claim 49 under 35 U.S.C. 103(a) as being unpatentable over Mori et al in view of David et al, and further in view of Zuo et al, Applicant's arguments filed 9/27/2010 have been fully considered but they are not persuasive.

The response asserts that the Examiner has failed to show how David and Zuo remedy the deficiencies of Mori with respect to independent claim 46. Thus, the response asserts that

claim 49 is patentable over Mori, David and Zuo for the reasons set forth above with respect to independent claim 46.

This argument is not found persuasive for the reasons set forth above with regard to the rejection of claim 46.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

With respect to the rejection of claims 52-54 under 35 U.S.C. 103(a) as being unpatentable over Mori et al in view of David et al, and further in view of Rasochova et al, Applicant's arguments filed 9/27/2010 have been fully considered but they are not persuasive.

The response asserts that the Examiner has failed to show ho David and Rasochova remedy the deficiencies of Mori with respect to independent claim 46.

This argument is not found persuasive for the reasons set forth above.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is (571)272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Jennifer Dunston/
Primary Examiner
Art Unit 1636